GENES INVOLVED IN THE MOLECULAR PATHWAYS OF TUMOR SUPPRESSION AND/OR RESISTANCE TO VIRUSES

INSCI)

The present invention relates to the revelation of genes involved in the molecular pathways of tumor suppression and/or resistance to viruses.

BACKGround of the Invention / Field of the Invention

The present invention has been made possible by the isolation of cDNA corresponding to messenger RNAs expressed or repressed during tumor suppression and/or during the process of apoptosis induced by the p53 suppressor gene.

Description of the Art

One of the most important suppressor genes involved in apoptosis is the p53 gene. When functioning normally, this gene controls cell growth and the process of apoptosis; in particular, it is this gene which blocks cell growth and which must induce the apoptotic process in order to avoid the development of a cancer. It has thus been demonstrated that mice nullizygous for p53 are much more sensitive to the formation of tumors. The fact that, in cancers, the p53 gene is very often modified and leads to the production of proteins incapable of vehicling the message of apoptosis has also been demonstrated.

It is this particularity which has been used in the context of the present invention.

Specifically, the present invention is based on the observation that it is not possible, or at least that it appears to be very difficult, to set up a direct substitution therapy when there is dysfunctioning of the p53 gene. Specifically, p53 which is mutated, as it is in cancer, will annul the physiological effect of normal p53.

It has therefore been necessary to abandon, at least initially, the idea of a substitution therapy acting directly on p53.

The present invention has, therefore, endeavored to study the genes located upstream and downstream of p53 in order to bypass the difficulty mentioned above.

In order to isolate the genes activated or

inhibited by normal p53 (wild-type p53), an overall combing of gene expression has been carried out in a malignant line (K562) and a derived cell (KS) in which the malignant phenotype is suppressed, more particularly in a cell expressing p53 which is functionally normal (KS) and in a cell not expressing p53 (K562). Comparison of the genes expressed (messenger RNAs expressed in the two types of cell) has made it possible to reveal genes expressed differentially, i.e. expressed in one of the cells whereas they are not expressed in the other (the genes may be activated or inhibited).

It is easily deduced therefrom that these genes are involved in the cancerization process, in one case by their absence and, in the other case, by their presence.

The method used for this differential study is the method described in 1992 by Liang and Pardee (Differential display of eukaryotic mRNA by means of a polymerase chain reaction).

Summary of the Invention

The approach to the problem according to the present invention has made it possible to isolate sequences directly linked to a function. Consequently, unlike the random sequencing of ESTs, the sequences are sequences the function of which is known and which are involved in the process of suppression of the malignant phenotype and/or of apoptosis induced by the p53 suppressor gene, and/or in resistance to viruses.

Thus, the present invention relates to novel sequences and the genes comprising them, and to the use of these sequences, both at the diagnostic and at the therapeutic level, just as for producing models intended to test anticancer and antiviral products.

The present invention relates, first of all, to a nucleotide sequence corresponding to a gene comprising:

(a) a sequence according to one of the SEQ IDs 1 to 15 or an equivalent gene which comprises:

(b) a sequence which hybridizes with one of the sequences according to (a),

(c) a sequence which has at least 80% homology with (a) or (b), or

(d) a sequence which encodes a protein encoded by a gene according to (a), (b) or (c), or an equivalent protein,

and to their use in particular in cancer suppression and/or resistance to viruses and in therapeutic monitoring.

It should be recalled that sequences 1 to 15 constitute only a part of the genes implicated, but that the present invention covers both the nucleotide sequence corresponding to the whole gene and fragments of this gene, in particular when they encode an equivalent protein as will be described hereinafter.

The nucleotide sequences can be both DNA sequences and RNA sequences, or sequences in which some of the nucleotides are unnatural, either in order to improve their pharmacological properties or in order to enable their identification.

The sequences mentioned in (b) are essentially the entire or partial complementary sequences (in particular for the cases mentioned above).

Thus, the invention also relates to the nucleotide sequences of the genes which have strong homology with the genes mentioned above, preferably greater than 80% homology on the essential portions of said genes, namely in general at least 50% of the sequence, preferably the homology will be greater than 90% on these portions.

Finally, when said genes encode a protein, the present invention also relates to the sequences encoding the same protein, taking into account the degeneracy of the genetic code, but also the equivalent proteins, i.e. the proteins producing the same effects, in particular the deleted proteins and/or the proteins which have undergone point mutations.

Detailed Description of the Invention

The sequences according to the present invention are more particularly the sequences which are induced or inhibited during cellular apoptosis, in particular those induced by p53 and/or p21 and/or TSAP3 (HUMSIAH) and/or antisense-TSIP2 (antisense-PS1). In other words, these sequences correspond to genes the cellular expression of which is activated by one at least of the transfectants chosen from the group comprising the p21 transfectants, the TSAP3 transfectants and the antisense TSIP2 transfectants.

Said genes are grouped together as TSAP or "Tumor Suppressor Activated Pathway" and termed from TSAP 9 to TSAP 22, corresponding to SEQ IDs 1 to 14, and as TSIP or "Tumor Suppressor Inhibited Pathay", and termed TSIP 3, corresponding to SEQ ID 15.

The characteristics of the sequences are given in the tables appended herein.

The nucleotide sequences corresponding to the TSAP genes are sequences which are expressed during the process of apoptosis, whereas when they are not expressed, the process of oncogenesis continues. It is therefore advantageous:

- to detect any abnormality in the corresponding gene, which abnormality may lead to greater susceptibility to oncogenesis, and
- to be able to provide a replacement therapy.

It should, moreover, be recalled that these genes may be involved in processes other than the processes of tumor suppression; specifically, p53 is in some ways the guardian of the integrity of the genome and under these conditions the TSAP or TSIP genes are doubtless also involved in this control function. It is, therefore, all of the possible modifications of the genome which may be liable to the detection and to the therapy above. On the other hand, the TSIP genes are expressed during oncogenesis and this expression is decreased, or even inhibited, during

apoptosis and tumor suppression; it is therefore, in this case as well, advantageous to detect the possible abnormality of the TSIPs and also to provide an inhibition/blocking therapy.

The replacement therapy may be carried out by gene therapy, i.e. by introducing the TSAP gene with the elements which enable its expression in vivo. The principles of gene therapy are known. Specific viral or nonviral vectors can be used, for example adenoviruses, retroviruses, herpesviruses or poxviruses. Most commonly, these vectors are used in defective forms which will serve as vehicles for TSAP expression with or without integration. The vectors can also be synthetic, i.e. mimic viral sequences, or consist of naked DNA or RNA according to the technique developed in particular by the company VICAL.

In most cases, it will be necessary to provide targeting elements which ensure tissue- or organ-specific expression; specifically, the activation of an uncontrolled phenomenon of apoptosis cannot be envisaged.

The present invention relates, therefore, to all of the vectors described above.

The present invention also relates to the cells transformed with an expression vector as described above, and to the protein which can be obtained by culturing transformed cells.

The expression systems for producing proteins can be both eukaryotic systems, such as the vectors above, and prokaryotic systems in bacterial cells.

I. [sic] one of the advantages of the present invention is that it has demonstrated the involvement of several genes in apoptosis; thus, the overexpression of one of the genes by gene therapy can, for some of them, drive to apoptosis only the cells in which other disturbed genes are already expressed, i.e. malignant cells.

The present invention also relates, as a medicine, to a compound which ensures the cellular expression of at least one of the nucleotide sequences above when it is induced during apoptosis and/or tumor suppression, in particular TSAP 9 to TSAP 22 genes, or conversely, which ensures the inhibition of the cellular expression of at least one cellular sequence as described above when it is inhibited during apoptosis and/or tumor suppression, in particular TSIP 3. It may, for example, be an activated nucleotide which ensures the blocking of the nucleotide sequence or be a monoclonal antibody directed against the protein(s) encoded by the nucleotide sequence.

Moreover, it is possible to envisage approaches other than gene therapy, in particular the use of nucleotide sequences in a sense or antisense strategy, i.e. which can block the expression of TSIP or, on the other hand, which act upstream, promoting the expression of TSAP.

It is also possible to envisage a direct replacement strategy by providing proteins corresponding to TSAP, or inhibitory antibodies corresponding to TSIP.

Finally, it is possible to envisage the use of nonprotein molecules, the activity of which will be to activate TSAP or to mimic the action of its expression product, or to inhibit TSIP or to block the action of its expression product.

These products can be easily tested on the modified cells which are described in the examples, by introducing the products to be tested into the cell culture and detecting the appearance of the apoptotic phenomenon.

In the DNA, RNA or protein strategies, the products are of course developed as a function of the sequences which are described.

The present invention relates, in particular, to the use of the medicines above as an anticancer agent.

However, the product of the TSAP 9 to 22 and TSIP 3

genes is also useful as an antiviral agent, as will become apparent upon reading the example.

The present invention also relates, therefore, to the use of the medicines above as an antiviral agent.

In addition, the present invention relates, as a diagnostic agent for determining the predisposition to cancer, to all or part of the sequences according to the invention to be used as a nucleotide probe or as an amplification primer, but also, as a diagnostic agent for determining the predisposition to cancer, to an antigen corresponding to all or part of the proteins encoded by the sequence according to the invention, or the corresponding antibodies, optionally after culturing.

The diagnostic methods are known; they may be, for example, techniques for microsequencing the variable portions after isolation and optional amplification, or methods for RFLP-detection or for simple amplification in particular. Differential techniques can, in particular, make it possible to demonstrate the difference between the normal and abnormal TSAP (or TSIP).

The invention also relates to the models using the sequences above.

Moreover, it should be emphasized that the inventors have demonstrated, by extension of the sequences in accordance with the invention initially revealed, homologies shown by said extended sequences with sequences corresponding to known proteins.

More particularly, besides the homology of TSAP 9 with a mouse chaperonin containing the TCP-1 gene (9), the inventors have brought to light a strong homology shown by TSAP13 with the p40.5 subunit of the human proteasome (10, 11) and a strong homology shown by TSAP 21 with syntaxin 11 belonging to the group of SNARE proteins (12).

The chaperonins are involved in the process of protein folding and assembly in the eukaryotic cytosol.

They are suspected of slowing down this folding by trapping intermediates which otherwise would aggregate. Among the proteins on which the chaperonin containing the TCP-1 gene homologous to TSAP 9 would act, mention may be made of actin, tubulin and the capsid protein of the hepatitis B virus.

The proteasome, in the same way as ubiquitin, is the main component of the major proteolytic system responsible for the degradation of many intracellular proteins, including aberrant proteins resulting from mutations or from environmental stress. The p40.5 subunit of the human 26S proteasome has recently been revealed, as well as its homolog in yeast Nas7p. In humans, the mRNA corresponding to the abovementioned subunit is more particularly expressed in the pancreas, placenta, testicles, heart and skeletal muscle. It appears, moreover, that yeast cells deficient for Nas7p are particularly sensitive to heat stress. This contributes to the suggestion that the function of the 26S proteasome is degraded during a heat stress.

The SNARE (Soluble N-ethylmaleimide-sensitive factor-attachment protein receptor) proteins are proteins the differential expression and the associations of which are involved in the organization of the membrane compartments of cells. These proteins are specifically located in the region of the Golgi apparatus, of the endosomes and of the lysosomes, which suggests that they play a role in the regulation of membrane exchanges using these organelles. More particularly, syntaxin 11 is thought to be located in the post-Golgi region.

It would be advantageous to be able to determine whether the molecular pathways in which the sequences in accordance with the invention are involved have common points with the molecular pathways in which the abovementioned proteins are involved, which would make it

possible to envisage novel modes of action on the abovementioned sequences for, for example, therapeutic or diagnostic purposes.

Figure 1 represents the extended TSAP 13 sequence (SEQ ID No. 5). The underlined portion corresponds to the sequence as originally brought to light by the inventors. The bold characters correspond to the sequence having 100% homology with the p40.5 subunit of the 26S human proteasome.

Figure 2 represents the extended TSAP 21 sequence (SEQ ID No. 13). The underlined portion corresponds to the sequence as originally brought to light by the inventors. The bold characters correspond to the sequence having 100% homology with syntaxin 11 of the group of SNARE proteins.

Other characteristics of the invention will become apparent upon reading the example below.

MATERIALS AND METHODS

Cell cultures

K562, KS, KS2 and KS3 cells were used as models. The K562 line is a tumor line derived from a chronic leukemia of erythromyeloid type. It is characterized in particular by a Philadelphia chromosome which contains the translocation (9,22) in which there is a rearrangement of the bcr gene with the abl proto-oncogene. This line has, moreover, an abnormal karyotype and overexpresses the myc and pim-1 oncogenes. These lines are described in the reference A. Telerman et al.: A model for tumor suppression using H-1 parvovirus, Proc. Natl. Acad. Sci. USA. Vol. 90, pp. 8702-8706, September 1993.

In summary, a monoclone of K562 was infected with the H-1 parvovirus. This infection caused a massive death of the cell culture. After maintaining this culture for a period of two months, the KS clone was isolated. The same experiment carried out a second time provided, after three

months of incubation, the KS2 and KS3 clones.

Using the same approach, the inventors derived, from a population of U937 malignant cells, the US3 and US4 lines, which are resistant to the H-1 parvovirus and which show suppression of the malignant phenotype. These lines are described in reference (7).

M1 myeloid leukemia cells and M1 cells were stably transfected with a heat-sensitive mutant val 135 p53 (LTR6).

These cells are cultured on RPMI 1640 medium with 10% FCS, at 5% of CO_2 at 37°C (3). For the temperature modification, the cultures are placed in a second incubator at 32°C.

U937 line transfected with $p21^{WAF1}$: the complete coding portion of the cDNA of the $p21^{WAF1}$ gene was cloned into the vector pBK-RSV (Stratagene, La Jolla, California). 3.5 million U937 cells were transfected with 20 micrograms of DNA/30 micrograms of Lipofectin (Life Technologies).

The stable transfectants were selected using 1.5 mg/ml of G418 (Sigma). The characteristics of this line portray in particular a suppression of the malignant phenotype.

U937 line transfected with TSIP2 (PS1) in the antisense position: the complete coding portion of the cDNA of the TSIP2 (PS1) gene was cloned, in the antisense position, into the vector pBK-RSV (Stratagene, La Jolla, California). 3 million U937 cells were transfected with 20 micrograms of DNA/30 micrograms of Lipofectin (Life Technologies).

The stable transfectants were selected using 1.5 mg/ml of G418 (Sigma). The characteristics of this line, portraying in particular a slowing down of growth, activation of apoptosis and suppression of the malignant phenotype, have been described in reference (8).

U937 line transfected with TSAP3 (HUMSIAH): the

complete coding portion of the cDNA of the TSAP3 gene was cloned into the vector pBK-RSV (Stratagene, La Jolla, California). 3 million U937 cells were transfected with 20 micrograms of DNA/30 micrograms of Lipofectin (Life Technologies).

The stable transfectants were selected using 1.5 mg/ml of G418 (Sigma). The characteristics of this line comprise in particular activation of apoptosis and suppression of the malignant phenotype.

Study of the differential cDNAs

In order to carry out the tests under standard experimental conditions and to obtain total reproducibility of the results, the following modifications to the protocol of origin (1) were carried out:

PolyA+ mRNAs purified twice on an oligodT column using Fast Track (Invitrogen, San Diego CA) are always used. After reverse transcription (M-MLV Reverse Transcriptase, Gibco BRL) on 0.05 µg of polyA+ using 20 µM of each of the dNTPs (Boehringer-Mannheim), no added dNTP is added to the final PCR mixture. A "hot start" at 94°C for 5 minutes is carried out before the PCR (GeneAmp PCR system 9600 Perkin Elmer Cetus). The samples are cooled rapidly on iced water. A "touch down" (2) of 10 cycles of 50°C to 40°C is carried out (94°C 30 seconds - 50°C 1 minute - 72°C 30 seconds), followed by 35 cycles (94°C 30 seconds - 40°C 1 minute - 72°C 30 seconds) and a final extension of 5 minutes at 72°C. The PCR products are separated on nondenaturing 6% polyacrylamide gels (4). The gels are exposed without drying. Each differential presentation is carried out by comparing M1S6 and LTR6 at 37°C and after incubating the two cell lines at 32°C for 4 hours.

The differential presentation procedure is repeated in 3 different experiments in order to confirm total

reproducibility.

The differentially expressed bands are cut out of the gel, eluted and reamplified (1). The PCR products are subcloned using the TA-cloning system (Invitrogen, San Diego, CA), following the instructions provided.

For each ligation reaction, 10 recombinant clones are sequenced using the ABI automatic system.

RNA extraction, analyses and Northern blot probes

The total RNA is extracted with Trizol (Life Technologies). The poly1+ [sic] RNAs are prepared using the OligotexdT kit (Qjagen, CA). 30 µg of the total RNA or 2 µg of polyA+ RNA are separated on 1% agarose, 1 × MOPS/2% formaldehyde gel and transferred onto nylon membrane (Hybond N+, Appligène, France) as has been described previously (5). The Northern blots are hybridized with probes labeled with P^{32} on the TSAP and TSIP inserts, and washed as described previously (5). In order to verify the induction of the wild-type p53 function, the Northern blots are hybridized with a cyclin G probe (6). By way of control for the amount of mRNA loaded, the blots are hybridized with a GAPDH probe. Various Northern blots (Clontech CA) are used under identical conditions and hybridized for control with a β-actin probe. The Northern blots are exposed for 10 days at -80°C.

Example 1

The desired aim is to characterize the molecular pathways which lead to the suppression of cancer.

The following hypothesis was made to develop a model: if it was possible to select, from a tumor which is sensitive to the cytopathic effect of the H-1 parvovirus, the cells which were resistant, this resistance might be due to a change in their malignant phenotype. It was possible to demonstrate this for the KS cells selected from

the K562 erythroleukemia cells. Unlike the parental K562 line, the KS, KS2 and KS3 clones are resistant to the cytopathic effect of the H-1 parvovirus. In addition, the tumorigenicity of the KS, KS2 and KS3 cells is reduced by 90%, while, when cultured in soft agar, the tumorigenicity of these same KS lines in vivo when injected into Scid-Scid immunosuppressed mice is reduced. At the molecular level, it could be noted that this suppression of the malignant phenotype went hand in hand with a reexpression of the p53 suppressor gene.

15 cDNAs expressed differentially between the K562 and KS cells were isolated. TSAP 9 is homologous to the chaperonins.

Table 1 shows the molecules characterized, giving the primers and the sizes of the mRNAs detected by Northern blot.

Of these 15 molecules, all are induced in the KS cells, except TSIP 3, the expression of which is inhibited during the suppression of the malignant phenotype.

In transfection experiments, it was also possible to demonstrate that the resistance to the cytopathic effect of the H-1 parvovirus went hand in hand with an intact function of the p53 gene and that cells transfected with p53 mutants became sensitive to the cytopathic effect of the H-1 parvovirus.

The 15 molecules which we have isolated encode, therefore, genes whose overexpression (TSAP 9 - TSAP 22) or inhibition (TSIP 3) is associated not only with the suppression of cancer, but also with resistance to the H-1 parvovirus. Consequently, these genes encode molecules which are part of the molecular pathways of cancer suppression and are potential suppressor genes.

In order to more clearly define the p53/p21 activation pathways, the inventors have studied:

- the activation of these TSAPs/inhibition of

the TSIPs in the heat-sensitive p53 model developed in Moshe Oren,

- the activation of these TSAPs/inhibition of the TSIPs in the model in which U937 cells are transfected with the p21 gene,
- the activation of the novel TSAPs/inhibition of the TSIPs in the model in which U937 cells are transfected with the TSAP3 gene, and
- the activation of these novel TSAPs/TSIPs in the model in which U937 cells are transfected with the TSIP2 (PS1) gene in the antisense position.

Table 1 below reports results of differential expressions analyzed by Northern blot of the various probes (TSAP9-TSAP22, TSIP3) of the K562/KS model and other U937/US3-US4 models, i.e. in a model of tumor suppression in which the p21 gene is activated via the p53 independent pathway. These cDNAs are therefore activated in two different cellular systems of tumor suppression (the K562/K2 erythroleukemia model and the U937/US myelomonocytic model).

According to this table, it is noted that, in the majority of cases, the genes expressed differentially in the K562/KS model are also expressed differentially in the U937/US3-US4 model. Molecular machinery for tumor suppression therefore exists which is common to various types of cancer. This conclusion is also valid for the M1/LTR-6 model. It should be noted, in the latter case, that the absence of signals in certain TSAPs-TSIPs is probably due to the fact that the experiments were carried out in two heterologous systems (human probes on mouse RNA).

TABLE 1

CLONE WITH DIFFERENTIAL EXPRESSION	3′ AND 5′* PRIMERS	K562/KS cDNA PROBE	HOMOLOGY	K562/KS MODEL mRNA kb
TSAP 9	T11AA-9	K26 D3	Chaperonin◊	2.6
TSAP 10	T11AA-9	K25.0 A11		1.6
TSAP 11	T11AA-9	K25.0 B7	EST	2.9
TSAP 12	T11AA-9	K27.1 C7	EST	5.5
TSAP 13	T11AA-23	K25.1 F3	Proteasome°	1.8
TSAP 14	T11AC-5	K33.2 F10	EST	2.5
TSAP 15	T11AG-19	K22 E3	EST	1.6
TSAP 16	T11GC-2	K12.1 F5		2.8
TSAP 17	T11GC-12	K16.1 C7		1.8
TSAP 18	T11GG-5	K3.1 D2	EST	2.0
TSAP 19	T11GG-23	K5.2 E 10	EST	1.5
TSAP 20	T11GG-23	K5.1 A12		1.7
TSAP 21	T11GG-23	K5.1 A1	SNARE$^{\Delta}$	2.1
TSAP 22	T11GG-5	K3.1 A12	EST	2.8
TSIP 3	T11AC-5	K33.1 B11	EST	9.5

* the numbers and sequences of the primers in the 5′ position correspond to those reported by Bauer et al.

◊ HUMKG1DD human mRNA for the ORF (human equivalent of mouse chaperonin containing the TCP-1 gene (t-complex polypeptide)).

° p-40.5 subunit of the proteasome (Nas7p)

$^{\Delta}$ SNARE syntaxin 11

TABLE 1 (continued)

CLONE WITH DIFFERENTIAL EXPRESSION	U937/US3-US4 MODEL		CLONE WITH DIFFERENTIAL EXPRESSION	M1/LTR-6 MODEL	
	RESULT	mRNA kb		RESULT	MRNA kb
TSAP 9	DIFF. EXP.	2.0	TSAP 9	DIFF. EXP.	2.6
TSAP 10	DIFF. EXP.	1.6	TSAP 10	NO SIGNAL	1.6
TSAP 11	NO DIFF. EXP.	2.8	TSAP 11	NO SIGNAL	2.9
TSAP 12	NO SIGNAL	5.5	TSAP 12	NO SIGNAL	5.5
TSAP 13	DIFF. EXP.	1.5	TSAP 13	DIFF. EXP.	1.8
TSAP 14	DIFF. EXP.	2.8	TSAP 14	DIFF. EXP.	2.5
TSAP 15	DIFF. EXP.	1.8	TSAP 15	NO SIGNAL	1.6
TSAP 16	DIFF. EXP.	2.0	TSAP 16	DIFF. EXP.	2.8
TSAP 17	DIFF. EXP.	1.9	TSAP 17	DIFF. EXP.	1.8
TSAP 18	NO DIFF. EXP.	1.8	TSAP 18	DIFF. EXP.	2.0
TSAP 19	DIFF. EXP.	1.6	TSAP 19	NO SIGNAL	1.5
TSAP 20	DIFF. EXP.	1.9	TSAP 20	NO SIGNAL	1.7

TSAP 21	DIFF. EXP.	1.9
TSAP 22	DIFF. EXP.	2.6
TSIP 3	DIFF. EXP.	9.5

TSAP 21	DIFF. EXP.	2.1
TSAP 22	DIFF. EXP.	2.8
TSIP 3	NO SIGNAL	9.5

DIFF. EXP. = Differential expression
NO DIFF. EXPR. [sic] = No differential expression

Table 2 below summarizes the differential expression of certain TSAP and TSIP clones in various transfectant lines.

It emerges from this table that, in the majority of cases, the p21 transfectants, TSAP3 transfectants or antisense-TSIP2 transfectants are capable of activating the molecular machinery of tumor suppression common to the U937/US and K562/KS systems.

TABLE 2

CLONE	P21 TRANS-FECTANTS DIFFERENTIAL EXPRESSION	TSAP3 TRANS-FECTANTS DIFFERENTIAL EXPRESSION	ANTISENSE TSIP2 TRANS-FECTANTS DIFFERENTIAL EXPRESSION
TSAP9	YES	YES	YES
TSAP10	YES	YES	YES
TSAP11	NO	NO	NO
TSAP12	NO	NO	NO
TSAP13	YES	YES	YES
TSAP14	YES	YES	YES
TSAP15	YES	YES	YES
TSAP16	NO	YES	NO
TSAP17	YES	NO	NO
TSAP18	NO	YES	YES
TSAP19	NO	NO	NO
TSAP20	YES	NO	YES
TSAP21	YES	YES	YES
TSAP22	YES	YES	YES
TSIP3	YES	YES	YES

Table 3 below recapitulates the characteristics of differential expression of the cDNA clones by Northern blot.

TABLE 3

cDNA clones	MRNA kb	HOMOLOGY	K562/K [sic]	U937/US	U937 p21	U937 AS PS1	U937 SIAH/ TSAP3
TSAP9	2.6	Chaperonin ◊	D	D	D	D	D
TSAP10	1.6	EST	D	D	D	D	D
TSAP11	2.8	EST	D	N	N	N	N
TSAP12	5.5	EST	D	N	N	N	N
TSAP13	1.8	Protea-some°	D	D	D	D	D
TSAP14	2.5	EST	D	D	D	D	D
TSAP15	1.6	EST	D	D	D	D	D
TSAP16	2.5	NO	D	D	N	N	D
TSAP17	1.8	NO	D	D	D	N	N
TSAP18	2.0	EST	D	N	N	D	D
TSAP19	1.5	EST	D	D	N	N	N
TSAP20	1.7	NO	D	D	D	D	N
TSAP21	2.1	SNARE$^{\Delta}$	D	D	D	D	D
TSAP22	2.6	EST	D	D	D	D	D
TSIP3	9.5	EST	D	D	D	D	D

D: Differential expression

N: No differential expression

◊: Chaperonin containing the TCP 1 gene

°: p40.5 subunit of the proteasome (Nas 7p)

$^{\Delta}$: SNARE syntaxin 11

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